IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Remacle et al.
Appl. No.	:	09/574,626
Filed	:	May 19, 2000
For	:	METHOD FOR THE IDENTIFICATION AND/OR THE QUANTIFICATION OF A TARGET COMPOUND OBTAINED FROM A BIOLOGICAL SAMPLE UPON CHIPS
Examiner	:	Zhou, S.
Group Art Unit	:	1631

DECLARATION UNDER 37 C.F.R §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. This Declaration is being submitted to demonstrate that as of 1999 the use of silver enhancement for *in situ* localization of target molecules was thought to result in variable sizes of precipitates, to produce background signals, to be non-quantitative. This Declaration is also being submitted to demonstrate that the presently claimed invention provides unexpected advantages.

2. I am not an inventor on the above-identified patent application and I am familiar with the specification and the claims at issue.

3. I have extensive experience with the utilization of metal enhancement in the context of *in situ* immunostaining of target molecules in cells. My Curriculum Vitae is submitted herewith as Exhibit I. In particular, I had been utilizing such technology for many years prior to 1999 as

evidenced by my publication of approximately 5 articles in which such technology was used prior to 1999. In addition, after 1999, I published many articles in which such methodology was utilized. In addition, I have experience with both fluorescence based microarrays and with the use of catalytic reduction of metals to detect target molecules on microarrays. I have used fluorescence-based microarrays in experiments for detecting differential gene expression in dynorphin knock-out mice. I am currently independently testing the Eppendorf's **Silverquant®** technology in gene expression **DualChip®** human hepato and **DualChip®** human general microarrays made by Eppendorf Array Technologies, the assignee of the above-identified application. I used the **Eppendorf Silverquant®** detection and scanning system for silver enhancement of targets bound to microarrays to detect the differential expression of nucleic acids associated with Alzheimer's disease. I am also testing a fluorescence detection on the **DualChip®** using the Affymetrix 428 array scanner. My report regarding the results of the testing is attached herewith as Exhibit II.

4. I am not affiliated with Eppendorf Array Technologies (the assignee of the above-identified application), although I am independently testing the **DualChip®** human hepato and **DualChip®** human general arrays in my laboratory as stated above. I will not financially benefit from the issuance of the above-identified application as a U.S. patent. I was, however, paid a wage of $250/hour for my expert services. In particular, this wage was paid to compensate me for the time I spent reading the specification and claims of the subject patent application, reading the Lockhart and Hacker references, and formulating the opinion expressed herein. Payment of this wage was not contingent on any particular outcome.

5. Gold and/or silver staining had been used in the context of tissue staining for as long as 20 years. Microarray technology was developed more than 15 years ago. However, rather than using catalytically generated metallic precipitates as in the present invention, prior to the present invention those working in the microarray field used fluorescence to detect target molecules bound to the microarray.

6. The use of metallic precipitates generated by catalytic reduction provides important advantages over alternative techniques. I have performed experiments using both the fluorescence microarrays and the arrays according to this invention. Side-by-side comparison of the two methods showed that the arrays according to this invention are more user-friendly, they

are easier to use and provide the results faster than the fluorescence microarrays. In particular, the arrays according to the present invention are more user-friendly because the **Silverquant**®detection system for gene expression microarray is very easy to handle, the **Silverquant**® scanner is very robust, the scan and analysis software are well designed, the presentation of the results is excellent. In addition, the **Silverquant**® scanning and analysis procedures are easier and faster than the fluorescence counterpart. The whole procedure of using the arrays of the present invention can be done in a laboratory setting requiring only technical skills of a technician, while the fluorescent microarrays require using a very cumbersome fluorescent reader which is operated by an expert. As a result, the use of the arrays of the present invention is also less expensive than the use of the fluorescent microarrays. In addition, the robustness of the entire system is better than fluorescence. In particular, we observed a lower background, higher signal intensity, higher signal-to-noise ratio and better reproducibility.

7. In addition to the unexpected advantages of using metallic precipitates generated by catalytic reduction to detect target molecules bound to microarrays, as of 1999, the literature relating to the use of silver enhancement in the context of *in situ* localization of target molecules in cells and my personal experience in this field indicated that the use of silver enhancement for *in situ* localization of target molecules resulted in variable sizes of precipitates, produced background signals, was non-quantitative. Even to this date, in the context of *in situ* localization of target molecules in cells, silver staining is often associated with higher variability than fluorescence. One drawback of using silver enhancement in the context of *in situ* localization of target molecules in cells is that, unlike fluorescence based methods, it lacks the capacity for multiple labeling. Such limitations would suggest that silver enhancement would not be suitable for detecting target molecules bound to microarrays, because microarray analysis requires consistent precipitate sizes to enable accurate quantitation of target molecules and comparison of targets bound to different portions of an array or to different arrays.

8. In conclusion, as of 1999, the use of silver enhancement for *in situ* localization of target molecules was thought to result in variable sizes of precipitates, to produce background signals, to be non-quantitative. In addition, the claimed invention possesses unexpected advantages which are not suggested by the combination of the cited references.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: ___12/21/06________________ By: ________________
Dr. Guoying Bing

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121406

EXHIBIT 1

CURRICULUM VITAE

Guoying Bing, M.D., Ph.D.

Associate Professor, Department of Anatomy & Neurobiology
University of Kentucky, School of Medicine

PERSONAL DATA

310 Davis Mills Building
Department of Anatomy & Neurobiology
University of Kentucky School of Medicine
Lexington, KY 40536-0298
E-mail: gbing@uky.edu
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543 Lake Tower Dr. Unite 133
Lexington, KY 40502
(859) 492-8000

EDUCATION

Doctorate of Philosophy, Anatomy and Neurobiology — October, 1988
Advisor: Dr. Don Gash
University of Rochester, Rochester, NY

Doctor of Medicine (equivalent) — September, 1977
Jilin Medical College, Jilin, China

PROFESSIONAL EXPERIENCE AND ACADEMIC APPOINTMENTS

2000-present **Associate Professor**, Department of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY

1991-present **Visiting Professor**, Beijing Institute of Neuroscience, Beijing, China

1997-2000 **Adjunct Professor**, Department of Cell biology, University of Oklahoma, Health Science Center.

1997-2000 **Assistant Member,** Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation

1993-1997 **Senior Fellow,** National Institute of Environmental Health Science

1991-1993 **Assistant Professor,** New York University Medical Center, Department of Psychiatry

1989-1991 **Postdoctoral Fellow,** NYU Medical Center, Department of Psychiatry

AWARDS AND OTHER PROFESSIONAL ACTIVITIES

2005	Charles T. Wethington Research Excellence Award; University of Kentucky
2000-2004	Charles T. Wethington Research Award Recipient; University of Kentucky
1982-1983	Fellowship for Chinese Graduate Student Study in USA, Chinese Government.
1983-1984	Fellowship from Educational Commission for Foreign Medical Graduate, USA
1985-1986	Research Fellowship, Society of Physiology

RESEARCH INTERESTS

Major research interests focus on the molecular and cellular mechanisms underlying the neurodegenerative diseases. Currently, there are three research projects are actively carried on in the laboratory: 1) the role of neuro-inflammatory processes in the etiology and pathophysiology of Parkinson's disease. 2). Long-term neuronal adaptation to excitatory neurotoxicity---Molecular cloning long-term, differential expressed genes in the hippocampus after KA-induced epileptic seizures; and 3). The role of xenobiotic metabolite enzymes in the central nervous system---The effects of environmental and endogenous toxins on neurodegeneration.

PATENT AWARDED

1. *Methods of using alpha 2 agonist for the treatment of neurodegenerative diseases.*
   Inventor: **Guoying Bing,** and Eric Stone. 1993.
   USA Patent Number: **5,252,816**

2. *A method for preventing and treating the degeneration of neurons.*
   Inventor, **Guoying Bing**, Naiying Zheng, Lei Jin, and Xin Lu, 1999
   USA Patent Number: **60,114,214.**

PATENT PENDING:

method of using PPAR gamma for preventing and treating the neurodegenerative diseases.
Inventor, **Guoying Bing** and Randy Hunter, 2005

GENE BANK SUBMISSION

1. *Molecular cloning of a new gene for Fos-related antigen (FRA) in the kainic acid treated hippocampus.*
   Submitted by: **Guoying Bing,** Qiping Qi, Zhihuei Feng and Jau-Shyong Hong.
   Accession Number: **U34932**

2. *Rat striatum genomic DNA of c-fos intron 3 and flanking cDNA sequence.*
Submitted by:, Zhihuei Feng, Kong L, Qiping Qi, No, S. Tiao, N., and **Guoying Bing**
Accession Number: **341647**

RESEARCH GRANTS

(*Principal Investigator unless otherwise noted*)

Active Supports

Guoying Bing, (P.I.) 9/17/01 - 9/16/06
NIH/NIMH F 30 Grant-MH65055
Project Title: Dynorphin in Age-related Impairment of Learning and Memory
The major goal of this grant is to investigate the mechanisms underlying age-dependent changes in neuronal or synaptic function and the potential role of dynorphin in mediating these changes.
Total Amount: $117,263

Guoying Bing, (P.I.) 7/1/03 – 6/30/08
NIH R01 Grant- NS044157
Project Title: COX-2 Deficient Mice are Resistant to MPTP Neurotoxicity
The goals of this study are to elucidate the changes in inflammatory processing affected by COX-2 deficiency, to explore the etiology and molecular mechanisms underlying Parkinsonian symptoms in the experimental MPTP model, and to develop novel therapeutic treatments for PD and other neurodegenerative diseases.
Total Amount: $1,425,000

Past Supports

Guoying Bing, (P.I.) 10/1/99 –9/30/03
US Army Medical Research Grant
Project Title: Protective Mechanisms of Nitrone Antioxidants in Kainic Acid Induced Neurodegeneration
Total Amount: $540,000

Guoying Bing, (P.I.) 7/1/98 –6/30/01
Principal Investigator for OCAST
Project Title: KA-induced gene expression in the hippocampus.
Total Amount: $150,000

Guoying Bing, (P.I.) 5/1/02

UK Microarray Pilot Program
Project Title: Differential Gene Expression in Hippocampus of Dynorphin Knockout mice.
Total Amount: $5,000

Guoying Bing, (P.I.) 12/1/99 - 11/ 31/04
NIH R01 Grant-NS39345
Project Title: Microglia Activation Induces Parkinsonism in rats

The major goal of this grant is to develop a new animal model that may be used in the development of novel therapeutic treatment for Parkinson's disease and other neurodegenerative diseases.

Total Amount: $830,000

Guoying Bing, (P.I.) 11/1/03
UK Microarray Pilot Program
Project Title: Microarray Detection of Patterns of Aging-Associated Genes Affected by Endogenous Dynorphin

The goal of this study is to investigate the mechanisms underlying age-dependent changes in neuronal or synaptic function and the potential role of dynorphin in mediating these changes; we propose to examine the effects of aging on memory in knockout mice lacking the coding exons for the precursor prodynorphin.

Total Amount: $5,000

Pending:

Guoying Bing, (P.I.) 4/1/04—3/31/06
Michael J. Fox Foundation
Project Title: COX-2 regulation of neuroinflammation in Parkinson's disease
Total Amount: $200,000

Guoying Bing, (P.I.) 6/1/04—5/31/06
Alzheimer Health Assistance Foundation
Project Title: Role of xenobiotic metabolism in Alzheimer's disease
Total Amount: $300,000

TEACHING EXPERIENCE

University of Kentucky College of Medicine, Lexington, KY

2003	**ANA 534;** Human Gross Anatomy; *Lecturer & Lab Instructor*
2002	**ANA 534;** Human Gross Anatomy; *Lecturer & Lab Instructor*
2001	**ANA 534;** Human Gross Anatomy; *Lecturer & Lab Instructor*

Oklahoma University, Oklahoma City, OK

1999 **Neuroscience Methods,** *Lecturer*

University of Rochester, Rochester NY

1987 **ANA 531;** System Neuroscience; *Lab Instructor*

Jilin Medical College, Jilin, China

1981 **Human Gross Anatomy**; *Lecturer & Lab Instructor*

GRADUATE STUDENTS

Thesis Advisor

Current MD., Ph.D. or Ph.D. candidates

Xuan Nguyen,	MD. Ph.D. candidate	1999-
Bin Xing	Ph.D. Candidate	2003-
Dong-Young Choi	Ph.D. Candidate	2004-

Past MD., MS. or Ph.D. candidates

Rattanavijit Vijitruth	**Ph.D.**	2001-2006
Randy Hunter	**MS**	2004-2006

Supervisor/Advisor

Undergraduate or graduate students

Raha Neal,	MD., Ph.D. Student 1999-2000
Candice Turner,	Bio 395undergraduate student 2001
Monica	Bio 395undergraduate student 2001

Current Postdoctoral Fellows

Mei Liu, **MD,** 2001-

Past Postdoctoral Fellows	Current Position
Deanna McCullers, Ph.D. 2002-2005	Technique writer
Yi Zhang, M.D. 1991-1993	Editorial assistant, Society of Physiology
Lei Jin, Ph.D. 1995-1999	Professor, Peking Union Medical University
Lingling Zhao, M.D., 1999-2001	Professor, Hunan Medical University
Toyoko Arimoto, Ph.D., 1999-2002	Staff Fellow, NIH
Anyang Sun, Ph. D., 1999-2002	Research Associate, Harvard Medical School

Professional trainees

Hyoung-Chun Kim, Ph.D., 1994-1996	Professor, Kangwon National University,

	Korea
Qiping Qi, Ph.D., 1995-1996	Director, Institute of National Academy of Preventive Medicine, China
Xianxi Liu, M.D., 1997	Professor, Shandong Medical University
Yahui Qi, M.D., 1998	Professor, Capital University of Medical Science, Beijing

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INVITED LECTURES

1. "Cografts of Adrenal Medullary cells with Neurotrophic producing Cells" Veterans Administration Hospital, Bedford, MA 01730, 1987.

2. "Transplantation of Adrenal Medullary, Carotid Body Glomus Cells with C6 Glioma Cells into the rat brain" Department of Anatomy, Boston University School of Medicine Boston, MA 02118, 1987.

3. "Neurotransplantation: Present and Future" Capital Institute of Medicine, Beijing, China, 1988.

4. 4, "Animal models used in neurotransplantation" New York University, Medical Center, New York, NY 10016, 1991

5. "Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra" NIEHS/NIH, Research Triangle Park, NC 27709, 1993.

6. "Long-term genomic effects of administration of kainic acid in the rat brain" Centaur Pharmaceutical Inc., Sunnyvale, CA 94086, 1995.

7. "The regulation of the opioid peptide by seizure activities ----Role of long-term AP-1 transcription factors". Oklahoma Medical Science Foundation, City, OK 73104, December, 1996.

8. "The regulation of the opioid peptide by seizure activities ----Role of long-term AP-1 transcription factors". University of Oklahoma, Oklahoma Center for Neuroscience, Oklahoma City, OK 73104, January, 1997.

9. Capital University of Medical Science, Beijing, China. March, 1997.

10. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" Kangwon National University, Korea. April, 1997.

11. "Long-term gene induction in the hippocampus by excitatory amino acid----A PCR-selected subtractive cloning methods" Shanghai Medical University, Shanghai, China. September, 1998.

12. "Current trends in research for neurodegenerative diseases" Shandong Medical University, Shandong, China. September,1998

13. "Microglia mediated neuronal death----A new animal model for Parkinson's disease" National Institute of Radiation Research, Ciba, Japan. June, 1999.

14. “Microglia mediated neuronal death----A new animal model for Parkinson’s disease” Yamagata University, School of Medicine, Yamagata, Japan, June, 1999

15. “Recent development of Molecular biological techniques in Neuroscience Research”. Capital University of Medical Science, Beijing, China. March, July, 1999.

16. “Microglia mediated neuronal death----A new animal model for Parkinson’s disease”. University of Missouri-Kansas City, School of Pharmacy, Kansas City, MS, August, 1999.

17. “Direct Visualization of Neurofibrillary Pathology in Alzheimer’s Disease” Kangwon National University, Korea. June, 2001.

18. “Gene therapy in neurological disease”. Capital University of Medical Science, Beijing, China. June, 2001.

19. “Microglia-activation Induced Parkinsonism” Capital University of Medical Science, Beijing, China. June, 2002

20. “A new animal model for Parkinson’s disease: microglial activation” 4th Ilsong international Symposium on Aging and Neurodegenerative Diseases in Seoul, Korea. December 2002

21. “Inflammation induced neurodegeneration” Shandong Medical University, Shandong, China. September, 2003

22. “A single intrapallidal LPS injection induces Parkinsonism in rats: A new animal model for Parkinson’s disease” International symposium on Parkinson’s disease, Beijing, China, September 2004.

23. “Inflammation and Parkinson’s disease” College of Pharmacy, Kangwon National University, Korea. September, 2004.

24. “Animal Model of Parkinson’s disease” Capital University of Medical Science, Beijing, China. June, 2004

25. “Can inflammation induces Parkinson’s disease?” Chinese Academy of Medical Science, Peking Union Medical College. May 2006

26. Shandong University Medical School; Jinan University Medical School; Central South University, Xianya Medical school. May 2006

COMMITTEE & SERVICE

2000-present University of Kentucky Medical Research Advisory Committee
2000-Present Graduate Faculty Committee, University of Kentucky, Medical Center
1997-2000 Graduate Faculty Committee, University of Oklahoma Health Sciences Center
1997-2000 Fleming Scholar Select Committee, Oklahoma Medical Research Foundation

REVIEW ACTIVITY:

Grant Review:

Ad Hoc reviewer: Alzheimer's Association.
Member, NINDS Study Section ZRG1
Ad hoc Member, Department of Defense, Medical Research
Oversee reviewer, Chinese Academy of Science

Journal Review:

Brain Research; **Brain Research Protocol**
Neuroscience; **Neuroscience Letter**
Neurodegeneration **J. Neuroscience**
Free Radical Biology and Medicine

PUBLICATIONS:

1. Gash, D.M., Notter, M.F.D., **Bing, G.**, Kordower, J.F. **(1986)** Neural implants into primates: Studies employing differentiated neuroblastoma cells. *Cell and Tissue Transplantation into the Adult Brain* pp. 37.

2. Hansen, J.T., **Bing, G.**, Notter, M.F.D., Gash, D.M. **(1987)** Ultrastructure of striatal implants of adult adrenal chromaffin cells in unilateral 6-OHDA lesioned rats. *Anat. Rec.* 218:56A.

3. **Bing, G.**, Notter, M.F.D., Hansen, J.T., Gash, D.M. **(1988)** Comparison of adrenal medullary, carotid body and PC12 cell grafts in 6-OHDA lesioned rats. *Brain Res. Bull.* 20:399-406.

4. Hansen, J.T., **Bing**, G., Notter, M.F.D., Gash, D.M. **(1988)** Paraneuronal grafts in unilateral 6-OHDA lesioned rats: Morphological aspects of adrenal chromaffin and carotid body glomus cell implants. In: *Transplantation into Mammalian CNS* (D.M. Gash and J. R. Sladek, Jr., Editors) Elsevier, Amsterdam, *Prog Brain Res*, 78:535-542.

5. Gash, D.M., Notter, M.F.D., Hansen, J.T., **Bing, G.**, Okawara, S.H. **(1988)** Human organ donor adrenals: Fine structure, plasticity and viability. In: *Transplantation into Mammalian CNS* (D.M. Gash and J. R. Sladek, Jr., Editors) Elsevier, Amsterdam, *Prog Brain Res.* 78:559-565.

6. Kordower, J.H., **Bing, G.**, Fiandaca, M.S., Sladek Jr., J.R., Gash, D.M. **(1988)** Tyrosine hydroxylase-immunoreactivity somata within the primate subfornical organ: Species specificity. *Brain Res.* 461:221-229.

7. Hansen, J.T., **Bing, G.**, Notter, M.F.D., Gash, D.M. **(1989)** Adrenal chromaffin cells as transplants in animal models of Parkinson's disease. *J. Electron Microscopy Tech.* 12:308-315

8. **Bing, G.**, Notter, M.F.D., Hansen, J.T., Kellogg, C., Gash, D.M. **(1990)** Cografts of adrenal medulla with C6 glioma cells in rats with 6-OHDA induced lesions. *Neurosci.* 34:687-697.

9. **Bing, G.**, Filer, D., Miller, J.C., Stone, E.A. **(1991).** Noradrenergic activation of immediate early genes in rat cortex. *Molec. Brain Res.* 11:43-46.

10. Stone, E.A., Zhang, Y., John, S., **Bing, G. (1991)** C-fos response to administration of catecholamine into brain by microdialysis. *Neurosci. Lett.* 133:33-35.

11. **Bing, G.**, Chen, S., Zhang, Y., Hillman, D., Stone, E.A. **(1992)** Noradrenergic-induced expression of c-fos in rat cortex: neuronal localization. *Brain Res.* 140:260-264.

12. Stone, E.A., **Bing G.**, John S.M., Zhang, Y., Filer, D. **(1992)** Cellular localization of responses to catecholamine in brain tissue. *Prog. Brain Res.* 94:303-307.

13. Stone, E.A., John, S.M., **Bing, G.**, Zhang, Y. **(1992)** Studies on the cellular localization of biochemical responses to catecholamines in the brain. *Brain Res. Bull.* 29:285-288.

14. **Bing, G.**, Stone, E.A., Zhang, Y., Filer, D. **(1992)** Immunohistochemical studies of noradrenergic-induced expression of c-fos in the rat CNS. *Brain Res.* 592:57-62.

15. Stone, E.A., Zhang, Y., John, S., Filer, D., **Bing, G. (1993)** Effect of locus coeruleus lesion on c-fos expression in the cerebral cortex caused by yohimbine injection or stress. *Brain Res.* 19:181-185.

16. Stone, E.A., Manavalan, J.S., Basham, D.A., **Bing, G**. **(1994).** Effect of yohimbine on nerve growth factor mRNA and protein levels in rat hippocampus. *Neurosci. Lett.* 14:11-13.

17. **Bing, G.,** Zhang, Y., Watanabe, Y., McEwen, B.S., Stone, E.A. **(1994).** Locus coeruleus lesions potentiate neurotoxic effects of MPTP in dopaminergic neurons of the substantia nigra. *Brain Res.* 668:261-265.

18. Hiller, J., Zhang, Y., **Bing, G.**, Gioannini, T., Stone E., Simon, E. **(1994)** Immunohistochemical Localization of mu-opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu-opioid binding protein. *Neurosci.* 62:829-841.

19. McMillian, M., Kong, L.-Y., Sawin, S.M., Wilson, B., Das, K., Hudson, P., Hong, J.-S., **Bing, G. (1995)** Selective killing of cholinergic neurons by microglial activation in basal forebrain mixed neuronal/glial cultures. *Biochem. Biophys. Res. Commun.* 215:572-577.

20. Das, K.P., McMillian, M., **Bing, G.**, Hong, J.-S. **(1995)** Modulatory effects of [Met^5]- enkephalin on interleukin-1b secretion from microglia in mixed brain cell cultures. *J. Neuroimmuno.* 62:9-17.

21. Perez-Otano, I., McMillian, M., **Bing, G.**, Hong, J.-S., Pennypacker, K. **(1996)** Induction of NF-kB-like transcription factors in brain areas susceptible to kainate toxicity. *Glia.* 16:306-315.

22. **Bing, G.**, Wilson, B., McMillian, M., Feng, Z., Qi, Q., Kim, H., Wang, W., Jensen, K., Hong, J.-S. **(1996)** Long-term expression of Proenkephalin and prodynorphin in the rat brain after systemic

administration of kainic acid ——an *in situ* hybridization study. In *Neurodegenerative Disease,* ed. by G. Flskum, Plenum Press, pp 8-18.

23. **Bing, G.**, McMillian, M., Kim, H., Pennypacker, K., Feng, Z., Qi, Q., Kong, L.-Y, Iadarola, M., Hong, J.-S. **(1996)** Long-term expression of the 35-kDa fos-related antigen (FRA) in rat brain after kainic acid treatment. *Neurosci.* 73:1159-1174.

24. Kim, H., Pennypacker, K., **Bing, G.**, Bronstein, D., McMillian, M., Hong, J.-S. **(1996)** the effects of dextromethorphan on kainic acid-induced seizures in the rat. *J. Neurotoxic.* 17:375-386.

25. Kong, L.-Y., McMillian, M., **Bing, G.**, Hudson, P.M., Hong, J.-S. **(1996).** The effects of the HIV-1 envelope protein gp 120 on the production of nitric oxide and proinflammatory cytokines in mixed glial cell cultures. *Cell Immunol.* 172:77-83.

26. **Bing, G.**, Wang, W., Qi, Q., Feng, Z., Jin, L., Bing, R., Hong, J.-S. **(1997)** Long-term expression of Fos-related antigen and transient expression of FosB associated with seizures in the hippocampus and striatum. *J. Neurochem.* 68:272-279.

27. Kim, H., **Bing, G.**, Hong, J.-S. **(1997)** Dextromethorphan blocks opioid peptide gene expression in the rat hippocampus induced by kainic acid. *Neuropeptides.* 31:05-112.

28. **Bing, G.**, Wilson, B., Hudson, P., Jin, L., Feng, Z., Zhang, W., Bing, R. **(1997)** A single dose of kainic acid elevates the levels of enkephalins and activator protein-1 transcription factors in the hippocampus for up to 1 year. *Proc. Natl. Acad. Sci., USA.* 94:9422-9427.

29. Simpson, J.N., Zhang, W.Q., **Bing, G.**, Hong, J.-S. **(1997)** Kainic acid-induced sprouting of dynorphin- and enkephalin-containing mossy fibers in the dentate gyrus of the rat hippocampus. *Brain Res.* 747:318-323

30. Feng, Z., Zhang, W., **Bing, G.**, Hundson, P., Feng, W., Hong, J.-S. **(1997)** Characterization of the long-lasting activator protein-1 complex induced by kainic acid treatment. *Brain Res.* 770:53-59.

31. Chen, S., Ren, Y.Q., **Bing, G.**, Hillman, D.E. **(1998)** Transient c-*fos* gene expression in cerebellar development and functional stimulation. *Brain Res* 795:87-97.

32. Gupta, R.P., **Bing, G.**, Hong, J.S., Abou-Donia, M.B. **(1998)** cDNA cloning and sequencing of Ca^{2+}/calmodulin-dependent protein kinase II subunit and its mRNA expression in diisopropyl phosphorofluoridate (DFP)-treated hen central nervous system. *Mol Cell Biochem.* 181:29-39.

33. Kim H.C., **Bing, G,** Jhoo, W.K., Ko, K.H., Kim, W.K., Lee, D.C., Shin, E.J., Hong, J.S. **(1999)** Dextromethorphan modulates the AP-1 DNA-binding activity induced by kainic acid. *Brain Res.* 824:125-132.

34. Feng, Z., Chang, R.C., **Bing, G.**, Hudson, P., Tiao, N., Jin, L., Hong, J.S. **(1999)** Long-term increase of Sp-1 transcription factors in the rat hippocampus after kainic acid treatment. *Brain Res* 69:144-148.

35. Kim, H.C., Jhoo, W.K., Choi, D.Y., Im, D.H., Shin, E.J., Suh, J.H., Floyd, R.A., **Bing, G. (1999)** Protection of methamphetamine nigrostriatal toxicity by dietary selenium. *Brain Res.* 851:76-86.

36. Floyd, R.A., Robinson, K.A., Stewart, C.A., **Bing, G.**, Hensley, K. **(1999)** Neuroinflammatory events

and signal transduction processes are involved in neurodegeneration. In: Free Radicals in Brain Pathophysiology. (Cadenas, E., Packer, L, Poli, G., Ed.) pp. 109-126, Marcel Decker, NY.

37. Hensley, K., Floyd, R.A., Zheng, N.Y., Nael, R., Robinson, K.A., Nguyen, X., Pye, Q.N., Stewart, C.A., Geddes, J., Markesbery, W.R., Patel, E., Johnson, G.V.M., **Bing, G.** **(1999)** p38 Kinase is activated in the Alzheimer's disease brain. *J. Neurochem.* 72:2053-2058.

38. Kim, H.C., **Bing, G.**, Jhoo, W.K., Ko, K.H., Kim, W.K., Suh, J.H., Kim, S.J., Kato, K., Hong, J.S. **(2000)** Changes of hippocampal Cu/Zn-superoxide dismutase after kainate treatment in the rat. *Brain Res.* 853:215-226.

39. Kim, H.C., Jhoo, W.K., Ko, K.H., Kim, W.K., **Bing, G.**, Kwon, M.S., Shin, E.J., Huh, J.H., Lee, Y.G., Lee, D.W. **(2000)** Prolonged exposure to cigarette smoke blocks the neurotoxicity induced by kainic acid in rats. *Life Sci.* 66:317-326.

40. Kim, H.C., Jhoo, WK, **Bing G**, Shin, E.J., Wie, M.B., Kim, W.K., and Ko, K.H. **(2000)** Phenidone prevents kainate-induced neurotoxicity via antioxidant mechanisms. *Brain Res.* 874:15-23.

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EXHIBIT 2

Field Test

"Silverquant® meets DualChip gene expression microarrays"

Evaluation form

eppendorf
In touch with life

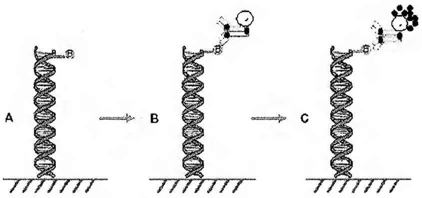
Aim

The intention of this field test is to gauge the compatibility of Eppendorf´s novel **Silverquant® technology** in gene expression DNA microarray experiments.

Description

Silverquant® is a colorimetric detection method that, by the amplification power of silver crystal precipitation on nanogold particles, enables the detection of biotin-labeled molecules on a microarray surface.

After hybridization of a biotinylated target molecule to the array surface a specific anti-biotin gold conjugate binds to the incorporated biotin. Subsequently, the silver is applied to the surface and precipitates on nanogold particles. Reduction of silver ions results in deposition of silver crystals on the surface of the gold particles. This high-contrast dark brown-to-black signals are generated in dependent of the amount of immobilized target molecules that can be detected by the Silverquant scanner. The gray level intensity is proportional to the quantity of bound target. Digitized images can be quantified with the Silverquant Software.



A. Specific binding of biotinylated products on the array.
B. Binding of Anti-biotin Conjugate gold.
C. Silver precipitation catalyzed by gold particles.

Figure 1: The nanogold particles are used as catalysts of the silver reduction

Contact Information

Dr. / Mr. / Mrs. / Ms: Bing and Dr Mei

Title:

FIRST NAME:

Last NAME:

POSITION:

INSTITUTION: University of Kentucky

DEPARTMENT:

PHONE 1:

PHONE 2:

FAX:

EMAIL:

May we contact you by email? ☐ YES ☐ NO

ADDRESS :

CITY:

STATE:

ZIP:

COUNTRY:

General Questions

1. What is your primary field of work (topic and organism)?

Study pathogens diseases on human brain tissue

2. Have you used arrays before?

Yesx ☐ No

If yes,
from which companies?

- Affymetrix ☐
- Clontech ☐
- MWG/Ocimum ☐
- Eppendorf x☐
- Agilent ☐
- GE Healthcare ☐
- PerkinElmer ☐
- CombiMatrix ☐
- Scienion ☐
- Memorec / Miltenyi ☐
- SuperArray ☐
- Home brew ☐
- self-spotting core facility ☐
- Other ☐, please specify: ____________________

3. What is your current cDNA labeling method?

- direct fluorescence labeling ☐
- indirect fluorescence labeling x☐
- Others ☐, please specify: ____________________

Technological Questions

1. Sample preparation

A. Which RNA extraction method did you use?

Product name: Trizol reagent from invitrogen

Commercial supplier: ______

Homebrew method: ______

B. How did you check the quality and quantity of the isolated RNA?

Eppendorf biophotometer
Gel for integrity

C. How much RNA did you use for the cDNA synthesis? (DualChip® human general experiments only)

6µg (easy to get from their samples)

eppendorf
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2. Handling Questions

A. Please rate the <u>complete system</u> (from cDNA synthesis to data analysis) on its ease of use?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Very easy

B. Please rate cDNA synthesis on its ease of use?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Very easy

C. Please rate the hybridization and silver staining procedures on their ease of use?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Very easy

Please specify critical steps

1. dispense hybridization mixture, bubbles, volume of mix is not perfect

2. remove frame because you have to be fast

D. Please rate the silver staining protocol on how easy it is to follow?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Very easy

E. Please rate the scanning procedure on its ease of use?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Very easy

F. Please rate the analysis procedure on its ease of use?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6 ☐ Very easy

G. Please rate the hybridization frame on its ease of handling?

Very difficult 1 ☐ 2 ☐ 3 ☐ 4x ☐ 5 ☐ 6☐ Very easy

H. What do you think is the most critical step of the experiment?

Isolation o RNA
Avoid RNAse contamination
Dispense the hybridization mix
Avoid touch the window

3. Instruction Manual

A. How would you rate the *"DualChip & Silverquant"* manual overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6☐ Excellent

Additional comments about the manual:

Confusion for silver step (5min+5min?)

B. Do you find the DualChip kit manual clear and easy to understand?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6☐ Excellent

Additional comments about manual:

One manual explaining from RT to analysis is perfect

4. Silverquant® Scanner

A. How do you rate the Silverquant scanner overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Additional comments about the scanner:

no

B. How satisfied are you with the scanning speed?

very unsatisfied 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ very satisfied

Additional comments about the scanning speed:

C. How satisfied are you with the design/footprint of the scanner?

very unsatisfied 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ very satisfied

Additional comments about the design / footprint:

D. How do you rate the handling of the slide holder?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Additional comments about the slide holder:

E. How do your rate the robustness of the scanner?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Additional comments about the robustness:

F. How do you rate the scanning process overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Additional comments about the scanning process:

eppendorf

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G. Was the barcode reading successful?

Yes x☐ (100%) No ☐

H. Did you use the Silverquant scanner manual ?

Yesx ☐ No ☐

I. What do you think are the strengths of the Silverquant scanner?

fast
efficient

J. What do you think are the weaknesses of the Silverquant scanner?

no

5. Silverquant® scan and analysis software

A. How do you rate the analysis software overall?

Silverquant scan software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ x6☐ Excellent

Silverquant analysis software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6☐ Excellent

Additional comments about either / both software platforms:

B. How do you rate the software on its ease of use?

Silverquant scan software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Silverquant analysis software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6☐ Excellent

C. How do you rate the process speed?

Silverquant scan software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

Silverquant analysis software

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x☐ Excellent

D. Did you use the Silverquant analysis software manual to start the process?

Yesx ☐ No ☐

6. Quantification of Silverquant® stained slides images

A. Did you get problems during automatic grid placement?

Yes ☐ Nox ☐

If yes, please specify:

B. Did you have to place the grid manually?

Yes ☐ Nox ☐

If yes, please specify:

C. Where all the spots been found?

yesx☐ No ☐

If yes, please specify:

7. Analysis of Silverquant® quantified data

A. How do you rate the complexity of loading quantified data files into analysis software?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

Additional comments on its complexity:

B. How do you rate the data processing?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

Additional comments on data processing:

C. How do you rate the normalization process?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Excellent

Additional comments on the normalization process:

D. How do you rate the presentation of the results?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

Additional comments on the presentation of the results:

E. Where there any options that you found were not useful?

yes ☐ nox ☐

If yes, what were they?

[illegible]

F. Did you find that the Silverquant analysis software lacked any function?

Yes ☐ Nox ☐

If yes, please specify:

G. Did you try to reopen analyzed data?

Yesx ☐ No ☐

Additional comments:

H. Did you use the Silverquant analysis software for quantifying your own arrays?

Yes ☐ Nox ☐

Additional comments:

[illegible]

I. How do you rate the quantification procedure of the Silverquant analysis software overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Excellent

Additonal comments on the overall procedure:

[illegible]

eppendorf

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8. Fluorescence analysis with DualChip® evaluation software

A. How do you rate the complexity of loading quantified data files into evaluation software?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6 ☐ Excellent

Additional comments on the complexity:

Do not know the orientation

B. How do you rate the normalization process?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6 ☐ Excellent

Additional comments on the normalization process:

C. How do you rate the presentation of the results?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6 ☐ Excellent

Additional comments on the presentation:

D. Did you find that the data presentation lacked any formats?

Yes ☐ Nox ☐

If yes, please specify:

E. Did you expect to find other functions that are not available in the DualChip evaluation software?

Yes ☐ Nox ☐

If yes, please specify:

G. Did you try to reopen analyzed data?

Yesx ☐ No ☐

If yes, please specify:

easy

H. How do you rate the quantification procedure of the evaluation software overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ Excellent

Additional comments on the overall procedure:

9. Results and quantity of Silverquant® experiments

Sample ID	Barcode and array position	Amount of starting material	Method of detection

Please save and send a copy of your scan pictures (incorporate barcode into the file name) of all DualChip arrays and quantified files performed with Silverquant on CD.

A. Please rate your first impression when scanning the slides.

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

B. Please rate the background.

- Homogeneity

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 x☐ 6 ☐ Excellent

- Background signal (2 first was horrible)

High 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Low

C. How would you rate the signal intensities of the DualChip / Silverquant system as compared to your current system (using an equal quantity fo starting material)

DualChip / Silverquant system is (choose one):
Higher x☐
Lower ☐
Equal ☐

D. How would you rate the reproducibility of this system overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

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E. How do your rate the quality of the DualChip results with Silverquant as compared to DualChip results with fluorescence?

Silverquant results 1 = insufficient ....6 = excellent							comparison to fluorescence results		
1	2	3	4	5	6		worse	equal	better
					x	Background			X
					x	Signal intensity			X
					x	Signal-to-noise ratio			X
					x	Reproducibility			X
				x		Dynamic range		x	
				x		Sensitivity		x	
				x		Ease of use hybridization & antibody incubation step		x	
					x	Ease of use scanning procedure			x
					x	Ease of use data analysis		x	
					x	Robustness of the entire system			x
				x		Ease of use entire system			x

I. How do you rate the Silverquant results in comparison to your expectations?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5x ☐ 6 ☐ Excellent

If less than 4, please specify:

J. What are your overall comments on Silverquant´s performance?

very good

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10. Packaging / Shipment

A. Do you find the labeling of the kits / vials forward straight?

DualChip kit	Yes x☐	No ☐
Silverquant detection kit	Yes x☐	No ☐

Additional comments:

sometimes we should write the total amount on vial

B. Would you expect to find problems due to the different storage conditions Internal Standard Mix, DualChip kit and Silverquant detection kit?

Yes ☐ Nox ☐

If yes, please specify:

C. Please enter any other comments concerning packaging below.

D. When did you receive the shipment?

Year / Month / Day

feb 06

call from custom for the second silverquant additive reagent

E. Did you receive the DualChip kits and Silverquant detection kit at 4°C?

Yesx ☐ No ☐

F. Please enter any other comments concerning shipment below.

11. General questions

A. How do you rate the Silverquant detection system for gene expression microarrays overall?

Poor 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6x ☐ Excellent

B. What do you think are the strengths of the system?

easy to handle
interesting because you detect interesting genes

C. Do you think there are any weaknesses with the system?

If yes, please specify:

I do not find any

D. Would you buy the Silverquant system for DualChip experiment?

Yesx ☐ No ☐
If you have more money, yes
Core facility should be interested in UKentuky
If no, please specify

E. Please enter any other comments below:

eppendorf

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